Structure determination of B_{12}-dependent ribonucleotide reductase from *Lactobacillus leichmannii*

M.D. Sintchak, G. Arjara, C.C. Lawrence, L. Shu, B.A. Kellogg, J. Stubbe,
and C.L. Drennan
Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Ave,
Cambridge, MA 02139, USA

INTRODUCTION

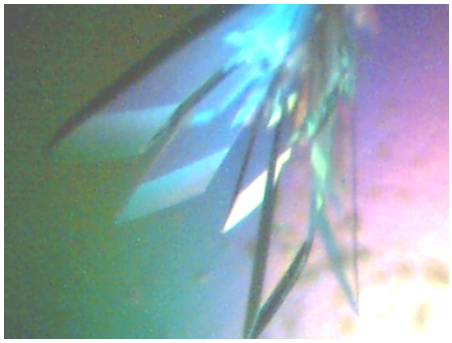
Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides in all organisms, and therefore play an essential role in nucleic acid metabolism. Their key role in DNA biosynthesis and repair makes them attractive targets for anti-tumor, anti-viral, and anti-bacterial therapies. In contrast to most enzymes central to metabolism, these reductases do not appear to be evolutionarily conserved, consisting of very different amino acid sequences and utilizing different metallocofactors to accomplish the same chemistry. Based on their differing cofactor requirements, the RNRs can be divided into four distinct classes. The well characterized metallocofactors include a diferric-tyrosyl radical (Class I), adenosylcobalamin (Class II), and a (FeS/S-adenosylmethionine) glycyl radical (Class III). In all cases, a radical generated by the particular cofactor is transferred to an active site Cysteine at a location distinct from the cofactor binding site. Thus, despite the wide array of cofactors nature has chosen to carry out this chemical reaction, it has been postulated that the actual mechanism of nucleotide reduction is conserved [1].

The underlying mechanism of allosteric regulation for these enzymes remains elusive. While a single enzyme can carry out the reduction of any one of the four nucloetides, the specifity is controlled by an allosteric effector at a different site. Because RNRs from *E. coli*, mammals, and herpesviruses are comprised of two homodimeric ($\alpha_2\beta_2$) subunits, structural studies of these enzyme-effector complexes have been hindered by the difficulties encountered in obtaining crystals of these multi-subunit assemblies. In contrast, ribonucleoside triphosphate reductase (RTPR, E.C. 1.17.4.2) from *L. leichmannii* is ideally suited for mechnistic studies since the active site, allosteric site, and cofactor site all must reside on a single polypeptide chain. While three-dimensional crystal structures are available for the R1 and R2 subunits of Class I RNR [2,3,4] and for the alpha subunits of the Class III enzyme [5], there is currently no structural information available for Class II RNRs utilizing coenzyme B_{12} as a cofactor, such as RTPR from *L. leichmannii*.

RESULTS

RTPR from *L. leichmannii* (738 amino acids, 82 kDa molecular weight) was cloned into *E. coli*, overexpressed, and purified as described previously [6]. Crystals of apo RTPR were grown

using standard hanging drop vapor diffusion at room temperature (Figure 1). Crystals typically appeared in one day and grew to an average size of approximately 80 x 160 x 400 μm in two weeks. For data collection, crystals were transferred with a nylon loop and flash-cooled directly in a nitrogen gas stream. The structure of apo RTPR was solved using multiwavelength anomalous dispersion (MAD) techniques after incorporation of selenomethionine into the protein. A three-wavelength inverse beam MAD dataset (Table 1) was collected on ALS beamline 5.0.2. All data were processed and scaled using Denzo and Scalepack [7]. Heavy atom site location and phase refinement were done using CNS [8]. The experimental anomolous difference Patterson map agrees quite well with the predicted Patterson map calculated based on 30 sites (Figure 2). After initial model building defined the location of all 36 methionine resdiues (for 4 molecules per a.s.u. and 9 SeMet residues per molecule), we noticed that a weak anomalous signal from the cysteine sulfur atoms was also present. These were included in the heavy atom model, bringing the total number of sites to 68 per a.s.u. Phasing statistics are shown in Table 2. Eventually, MAD phases to 2.5 Å were merged with higher resolution native data and extended to 1.8 Å resolution using solvent flattening and 4-fold NCS averaging in the CCP4 program dm [9]. A representative density map calculated using only experimental phases and data to 1.8 Å resolution is shown in Figure 3, along with electron density calculated at a later stage in refinement (Figure 4). We are currently completing the refinement of the apo enzyme structure and are working to obtain the structure of RTPR in complex with cofactor, substrate, and effector molecules bound.



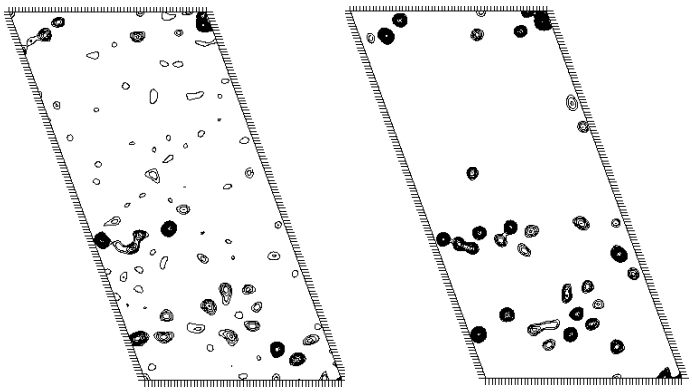


Figure 2: anomalous difference Patterson maps calculated from data (left) vs. predicted (right)

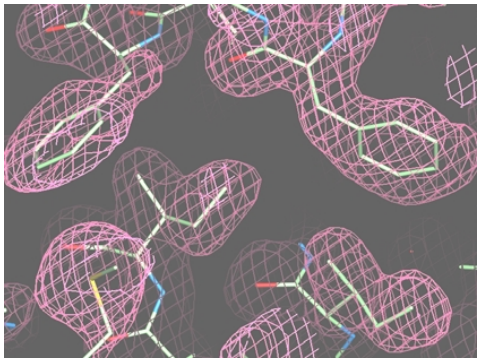


Figure 3: experimental phases at 1.8 Å (1.5σ contour)

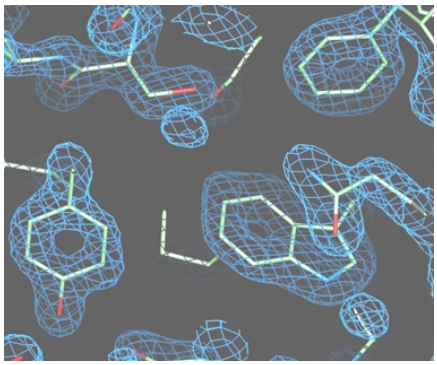


Figure 4: 2Fo-Fc density at 1.75 Å (3σ contour)

Table 1: Data Collection Statistics (MAD)

	λ1 (peak)	λ2 (edge)	λ3 (remote)
Wavelength (Å)	0.9797	0.9800	0.9537
Resolution (Å)	100 - 2.2	100 - 2.3	100 - 2.2
Rsym (%)	6.8 (28.7)	7.3 (31.7)	6.2 (23.9)
Total Observations	1049123	922025	1154666
Unique reflections	304627	268271	318229
Average Redundancy	3.4	3.4	3.6
<I/σ>	19.9 (3.9)	18.1 (3.5)	22.3 (5.0)
Completeness (%)	99.2 (98.1)	99.3 (98.2)	99.7 (99.1)

(NOTE: numbers in parenthesis above are for highest resolution shell)

Table 2: Phasing Statistics using Se-Met MAD Phasing for 68 sites total (36 Se + 32 Sulfur)

resolution range	500 - 2.5 Å
number of reflections used in phasing	207400
Final FOM (centrics)	0.82
Final FOM (acentrics)	0.65
Overall FOM	0.65

REFERENCES

1. Stubbe, J. *Proc. Natl. Acad. Sci USA*, **95**, 2723-2724 (1998).
2. Nordland, P., Sjoberg, B.-M. and Eklund, H. *Nature*, **345**, 593-598 (1990).
3. Uhlin, U., and Eklund, H. *Nature*, **370**, 533-539 (1994).
4. Logan, D.T., Su, X.-D., Aberg, A., Regnstrom, K., Hajdu, J., Eklund, H., and Nordlund, P. *Structure*, **4**, 1053-1064 (1996).
5. Logan, D.T., Andersson, J., Sjoberg, B.-M., and Nordlund, P. *Science*, **5**, 1499-1504 (1999).
6. Booker, S., and Stubbe, J. *Proc. Natl. Acad. Sci USA*, **90**, 8352-8356 (1993).
7. Otwinowski, Z., and Minor, W. *Methods Enzymol.*, **276**, 307 (1997).
8. Crystallography & NMR System (CNS). *Acta Cryst.*, **D54**, 905-921 (1998).
9. Collaborative Computational Project, Number 4. *Acta Cryst.*, **D50**, 760-763 (1994).

This work was supported by laboratory start-up funds from the Dept. of Chemistry at MIT.

Principal investigator: Catherine L. Drennan, Dept. of Chemistry, MIT, Cambridge, MA 02139, USA. Email: cdrennan@mit.edu. telephone: 617-253-5622.